Environmental Health Perspectives
Vol. 88, pp. 13–25, 1990

Cellular Responses to Oxidative Stress: The [*Ah*] Gene Battery As a Paradigm

by Daniel W. Nebert,* Daniel D. Petersen,* and Albert J. Fornace, Jr.†

A major source of oxidative stress in animals is plant stress metabolites, also termed phytoalexins. The aromatic hydrocarbon-responsive [*Ah*] gene battery is considered here as a model system in which we can study metabolically coordinated enzymes that respond to phytoalexin-induced oxidative stress. In the mouse, the [*Ah*] battery comprises at least six genes: two Phase I genes, *CYP1A1* and *CYP1A2*; and four Phase II genes, *Nmo-1*, *Aldh-1*, *Ugt-1*, and *Gt-1*. All six genes appear to be regulated positively by inducers such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and other ligands of the Ah receptor. In the absence of foreign inducer, the control of *Nmo-1* gene expression is independent of the control of *CYP1A1* and *CYP1A2* gene expression. The radiation deletion homozygote c^{14CoS}/c^{14CoS} mouse is lacking about 1.1 centiMorgans of chromosome 7. Although having no detectable *CYP1A1* or *CYP1A2* activation, the untreated c^{14CoS}/c^{14CoS} mouse exhibits markedly elevated transcripts of the *Nmo-1* gene and three growth arrest- and DNA damage-inducible (*gadd*) genes. These data suggest that the missing region on chromosome 7 in the c^{14CoS}/c^{14CoS} mouse contains a gene(s), which we propose to call *Nmo-1n*, encoding a *trans*-acting factor(s) that is a negative effector of the *Nmo-1* and *gadd* genes. The three other [*Ah*] battery Phase II genes behave similarly to *Nmo-1* in the c^{14CoS}/c^{14CoS} mouse. This coordinated response to oxidative stress and DNA damage, by way of the release of a mammalian battery of genes from negative control, bears an interesting resemblance to the SOS response in bacteria.

Introduction

From the beginning of life on earth, toxicity caused by atmospheric oxygen has been a major challenge to living organisms. It is well documented that in bacteria several global regulatory mechanisms exist for the coordinated expression of enzymes and proteins needed for cellular protection against damaging agents such as those producing oxidative stress (*1–4*).

The purpose of this review is to pull together a number of seemingly unrelated observations concerning cellular responses to environmental adversity. First, we define oxidative stress. Next, we discuss phytoalexins, plant stress metabolites that are a major source of oxygenated metabolites in the diet of animals. Next, we introduce the [*Ah*] battery, a group of at least six genes encoding metabolically coordinated enzymes that respond to phytoalexin-induced oxidative cytotoxicity; in addition, the Ah receptor appears to play a role in programmed cell death, or apoptosis. Finally, we suggest a possible link by which several genes in the [*Ah*] battery and several other DNA damage-inducible genes are regulated in coordination.

*Laboratory of Developmental Pharmacology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892.

†Radiation Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

Address reprint requests to D. W. Nebert, Department of Environmental Health, University of Cincinnati Medical Center, Cincinnati, OH 43267.

Oxidative Stress Defined

Oxidative stress is produced by dioxygen and its various reactive metabolic intermediates. The major causes of oxidative stress include ionizing irradiation, normal metabolism at 37°C, inflammation, metabolic reactions involving outer-shell electrons of transition metals, and foreign chemicals that enter the organism. A major source of oxidizing agents in the diet of animals is plant stress metabolites, also called phytoalexins.

Phytoalexins

Phytoalexins have been defined as "low-molecular-weight antimicrobial compounds that are both synthesized by, and accumulate in, plant cells exposed to microorganisms" (*5*). Presumably such stress metabolites are also important in protecting plants against insect and other animal predators.

The response by plants to pathogen attack is illustrated in Figure 1. Microbial elicitors of host-resistance responses include peptides and enzymes, fatty acids, polysaccharides, and glycoproteins. Elicitors (inducers) appear to be both exogenous and endogenous (*6*). The

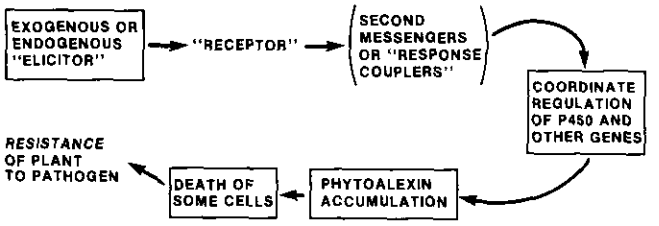

FIGURE 1. Sequence of events by which a plant responds to a pathogen's signal and develops resistance to that pathogen. Although mostly studied for microorganisms, the elicitor signal might arise from higher organisms, e.g., insects.

mechanism of transduction of these signals (i.e., receptors on the plant cell wall surface, intracellular second messengers, or other response couplers) is poorly understood. Ethylene biosynthetic metabolites and intracellular Ca^{2+} may be involved in signal transduction. The ultimate response to the elicitor is the accumulation of phytoalexins. Intracellular phytoalexins can be effective in killing the pathogens inside the plant cell. However, if the pathogen remains extracellular, as in the case of numerous bacterial and fungal infections, the death of some plant cells is required for the release of phytoalexins to kill the attacking pathogen.

Phytoalexins include phenylpropanoid-, terpenoid- and ethylene-derived chemicals, in addition to hydroxyproline-rich glycoproteins and proteinase inhibitors (*6*). This review will be limited to the former oxygenated metabolites. Chemical structures of representative phytoalexins are shown in Figure 2. From the chemical structures, it is clear that Phase I (P-450 and flavin-containing monooxygenases) enzymes in plants are necessary for the biosynthesis and degradation of most, if not all, of these phytoalexins. It should be emphasized that many, perhaps most, of these phytoalexins are toxic—not only to the invading pathogen but to animals ingesting the plants for food. Such toxicity might be manifested by the parent compound directly, or by a reactive metabolite formed in the invading pathogen (or in the animal that has eaten the plant). Although very few phytoalexins have been tested in animal cancer bioassays, about half of those examined (20 of 42) were found to be carcinogenic (*7*).

There are tens of thousands of different phytoalexins, and they are extremely abundant. Although only a few dozen stress metabolites might be present in each plant species, they usually account for 5 to 10% of the plant's dry weight (*7*). When plants are damaged or stressed (e.g., by being bruised, cut, peeled, marinated in salt or spices, heated or chilled) these phytoalexin levels may increase many-fold in several hours (*6*). Ingestion (mastication, swallowing, and gastrointestinal absorption) of plants causes these chemicals to be released into the animal.

A recently developed strain of celery is an interesting example of the interplay between phytoalexin-induced resistance to microorganisms and human disease (*8*). The usual concentration of the carcinogenic phytoalexin

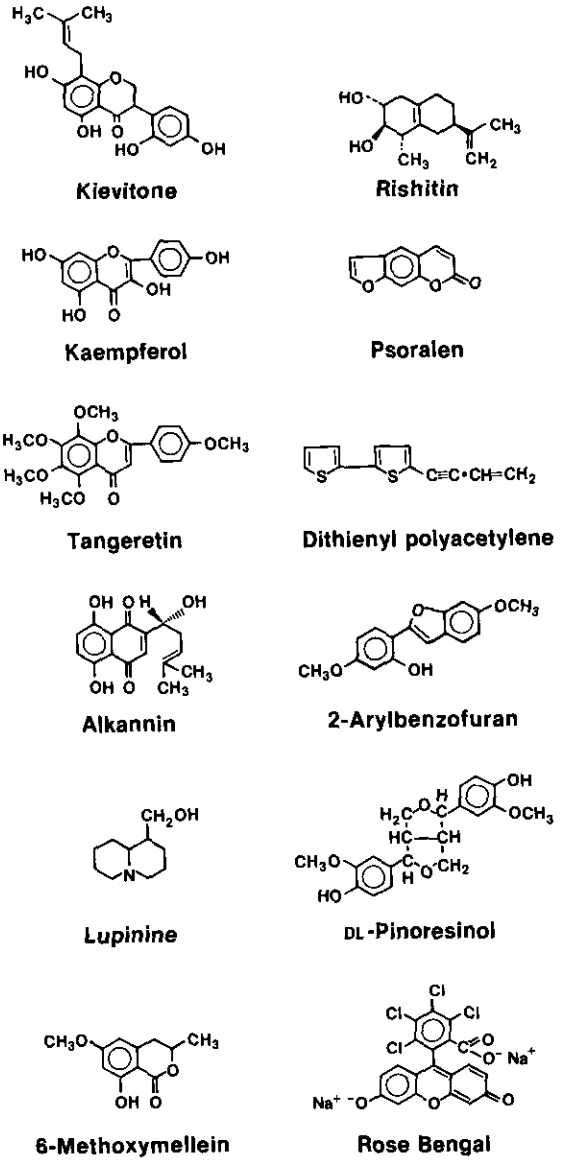

FIGURE 2. Chemical structures of 12 representative phytoalexins.

8-methoxypsoralen (and related psoralens) in celery is 900 ppb and is capable of producing dermatitis among celery handlers. A new variety of insect-resistant celery caused a greater incidence of dermatitis among produce and grocery workers and was found to have a 10-fold higher concentration of psoralens (9,000 ppb). As described recently by Ames and co-workers (*7*), it has become evident that the number and concentrations of "nature's own pesticides" (i.e., phytoalexins) may be a far greater risk to human health than man-made pesticides.

Cellular Responses to Quinones

Quinones are among the most electrophilic and therefore most reactive and toxic of the phytoalexins. The degree of quinone cytotoxicity varies greatly, depend-

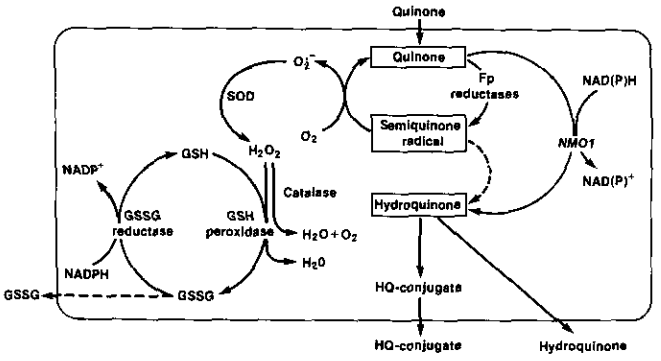

FIGURE 3. Diagram of the major pathways by which the cell responds to quinones. As described in the text, *Nmo-1* is the most important enzyme metabolizing quinones to hydroquinones by a two-electron reduction pathway. Superoxide dismutase (SOD) transforms O_2^- to hydrogen peroxide, which is then converted by catalase and glutathione (GSH) peroxidase to water and oxidized glutathione (GSSG), respectively. HQ, hydroquinone. Modified and redrawn from Orrenius (*9*).

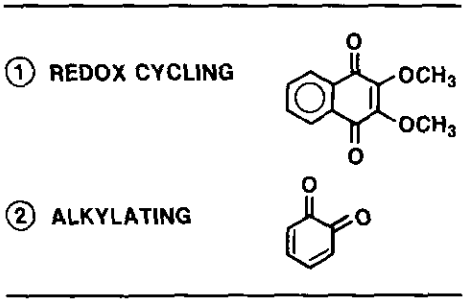

FIGURE 4. Chemical structures of representative quinones that act primarily by redox cycling and alkylation, respectively.

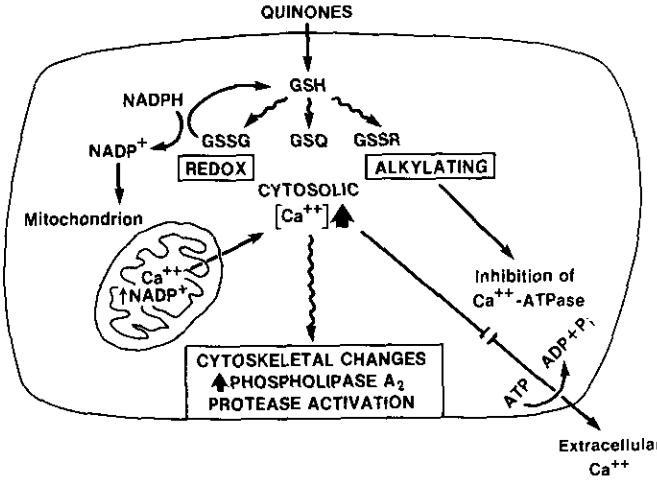

FIGURE 5. Schematic illustration of how quinones cause toxicity by both redox cycling and alkylation. GSQ, glutathione conjugate of quinone; GSSG, oxidized glutathione; GSSR, glutathione covalently bound to cellular macromolecules; P_i, inorganic phosphate. Modified and redrawn from Orrenius et al. (*11*).

ing upon whether the quinones are metabolized by one-electron or two-electron reduction pathways (Fig. 3). A one-electron reduction leads to the formation of semiquinone radicals, which can regenerate the quinone and form the superoxide anion radical O_2^- from dioxygen. The reaction can cycle repeatedly between the semiquinone radical and the starting quinone (Fig. 3); as redox cycling continues, this leads to cytotoxicity by way of dismutation of O_2^-, the formation of other reactive oxygen species and free radicals, depletion of reduced glutathione, and decreases in the endogenous NADPH pool. This recycling pathway can be circumvented by the two-electron reduction of quinones to hydroquinones by NAD(P)H:menadione oxidoreductase (NMO1) (also called DT-diaphorase and quinone reductase). These hydroquinones are usually less reactive, more easily conjugated with glutathione and glucuronide, and more easily excreted from the cell than are semiquinone radicals (*9*). Hence, elevated levels of the NMO1 enzyme would appear to protect the cell from quinone toxicity.

The elegant pioneering studies of Orrenius and coworkers have shown that, among the several one-electron reduction pathways, some quinones are more likely to undergo redox cycling, whereas others are primarily alkylating agents (*10*). For example (Fig. 4), *o*-benzoquinone is an extremely potent electrophile and, as such, is involved in both alkylation and redox cycling. In contrast, 2,3-dimethoxy-1,4-naphthoquinone undergoes redox cycling but not alkylation. Depletion of the endogenous NADPH pool during redox cycling leads to enhanced levels of $NADP^+$, which enters the mitochondrion and causes intramitochondrial Ca^{2+} to become cytosolic (Fig. 5). Inhibition of the Ca^{2+}-ATPase during quinone alkylation inhibits the release of Ca^{2+} from the cell, contributing further to elevated intracellular Ca^{2+} concentrations (*12*). Subsequently, other events include intracellular alkalinization, marked increases in phospholipase A_2 activity, activation of proteases, and toxic alterations in the cytoskeleton (*11*).

Apoptosis

Cytotoxicity is often associated with generalized chromatin condensation, DNA fragmentation, elevated cytosolic Ca^{2+} levels, intracellular alkalinization, surface membrane blebbing, and activation of an endogenous nuclear Ca^{2+}-dependent endonuclease (*11*). Interestingly, these same observations are also seen during a sequence of events described as programmed cell death or apoptosis (*11,13–15*). Apoptosis is known to occur during embryonic development, normal tissue turnover and hormone-dependent atrophy (Fig. 6). The changes in chromatin, as well as the DNA damage, probably reflect the activation of at least one endogenous Ca^{2+}-dependent endonuclease (*15*).

The Ah Receptor and Apoptosis

There are exogenous, as well as endogenous, stimuli that appear to cause apoptosis. One of many such ex-

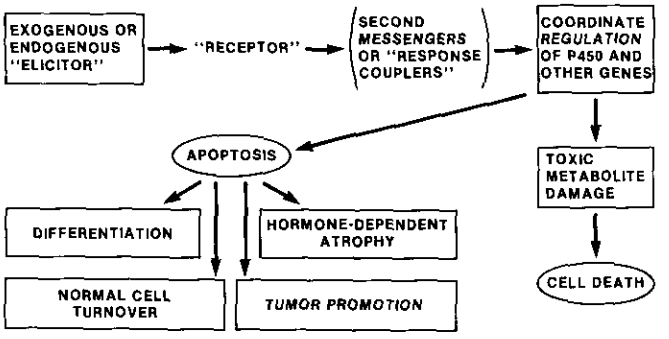

FIGURE 6. Sequence of events by which an animal responds to certain endogenous or exogenous signals that lead to programmed cell death (apoptosis) and nonspecific cell death. Plant phytoalexins are among the elicitors.

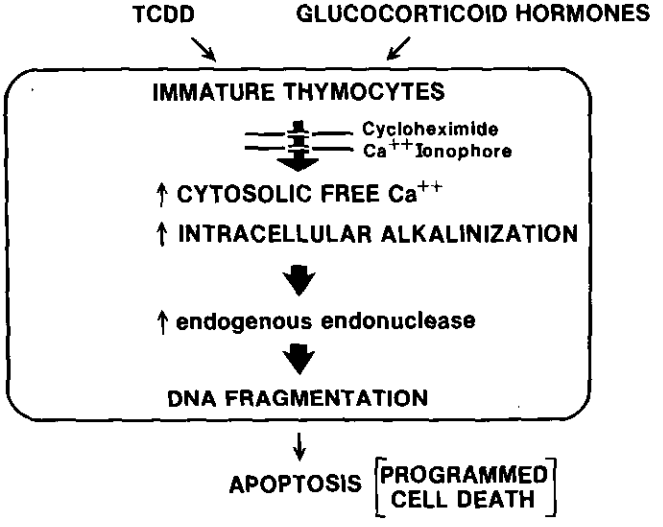

FIGURE 7. Hypothetical scheme by which both TCDD and glucocorticosteroids can cause apoptosis in immature thymocytes. The data are taken from McConkey et al. (*18*).

ternal signals is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The aromatic hydrocarbon-response (Ah) receptor (*16*) binds to TCDD and is believed to be a member of the steroid/retinoid/thyroid hormone superfamily (*17*). As shown in a recent fascinating study (*18*), TCDD and glucocorticoid hormones, both acting by way of their respective receptors, are capable of causing apoptosis in the immature thymocyte (Fig. 7). This suicide process can be blocked by cycloheximide and by the Ca^{2+} ionophore A23187, indicating that apoptosis is associated with new protein synthesis and elevated intracellular calcium levels (*18*). What do TCDD and glucocorticoid hormones have in common? It is intriguing that both are known to be potent tumor promoters (*19*), both activate specific P-450 genes (*16*), and both cause immunosuppression (*20*).

Role of Cytotoxicity in Tumor Promotion

How might TCDD be involved in apoptosis and tumor promotion? As we describe below, TCDD induces enzymes that generate mutagenic and toxic intermediates. These reactive oxygenated intermediates play an important role in tumor initiation as well as cytotoxicity, which is a necessary component of tumor promotion.

DNA damage is known to lead to adduct formation and mutation fixation, which are early critical events in the initiation of cancer. Although DNA adduct formation is often a prerequisite, during the progression of malignancy the process of tumor promotion is probably at least as important as the initial mutagenic event. Because tumor promotion has been experimentally difficult to study in most tissues, this cellular process has only recently begun to be understood (*21*).

The process of tumor promotion represents the clonal expansion of cells that have already undergone an initial mutagenic change (*21*). Included in this process is the killing of neighboring cells so that only a few cells might survive and multiply. Cytotoxicity is therefore of major importance and an essential component during tumor promotion. Interestingly, the process of tumor promotion (usually caused by exogenous stimuli) shares several characteristics with the process of apoptosis (caused by endogenous signals): elevated cytosolic Ca^{2+} levels, activation of the Ca^{2+}-dependent endonuclease, and intracellular alkalinization.

Tumor promoters such as serum growth factors, bombesin, plant lectins, and 12-*O*-tetradecanoylphorbol-13-acetate cause protein kinase C-dependent intracellular alkalinization (*22,23*), whereas Ha-*ras* expression does so by an as yet unknown protein kinase C-independent mechanism (*24*). Stimulation of the Na^+/H^+ antiporter (Na^+ is pumped in; H^+ out), and the resulting cytosolic alkalinization, has been observed in response to numerous growth factors and mitogenic agents and is viewed as either essential for or at least permissive of most if not all types of epithelial cellular proliferation (*22–24*). In almost every experimental system, amiloride and its derivatives can inhibit intracellular alkalinization and this proliferative process (*23*). Very recently, the amiloride-sensitive Na^+/H^+ antiporter protein from rabbit kidney has been purified (*25*). It will be interesting to clone and characterize the gene encoding this Na^+/H^+ transport protein.

The [*Ah*] Gene Battery

What might phytoalexin-induced oxidative stress and apoptosis have in common? Some insight might be gained through the use of an experimental system in which both processes are involved. Accordingly, the TCDD-inducible [*Ah*] gene battery is introduced here as a model system for understanding a particular set of metabolically coordinated enzymes that respond to oxidative stress. In addition, the Ah receptor plays some

role in apoptosis, and phytoalexins are believed to be among the ligands that bind to the Ah receptor.

During the past two decades, a group of six genes has been defined as the [*Ah*] gene battery (*16,26*). Inducers such as TCDD, benzo[*a*]pyrene, 3-methylcholanthrene, and β-naphthoflavone (Fig. 8) are known to bind to the Ah receptor with an apparent K_{diss} in the nanomolar to picomolar range. The endogenous ligand, if one indeed exists, is not known. There are similarities between the chemical structures of these ligands (Fig. 8), however, and those of several representative phytoalexins (Fig. 2). Hence, it is tempting to speculate that during evolution, the Ah receptor evolved in animals as part of a transducer pathway for detecting significant concentrations of toxic plant flavones and providing a beneficial response, i.e., the induction of enzymes specifically needed for detoxifying flavones and compounds of chemically related structure.

The inducer-receptor complex undergoes a temperature-dependent modification before gaining chromatin-binding capacity. The result is an Ah receptor-mediated positive transcriptional activation of each of the genes in the [*Ah*] battery (*16,26–29*). For some of these genes, a component of posttranscriptional regulation may also be present, such as has been characterized most completely for the mouse *CYP1A2* gene (*26*).

The TCDD-dependent Ah receptor-dependent induction in the mouse involves two Phase I genes, cytochrome P_1-450 (*CYP1A1*) and cytochrome P_3-450 (*CYP1A2*), and four Phase II genes, NAD(P)H:menadione oxidoreductase (*Nmo-1*), aldehyde dehydrogenase (*Aldh-1*), UDP-glucuronosyltransferase (*Ugt-1*), and glutathione transferase (*Gt-1*) (Fig. 9). Examples of the catalytic activities of each of these six genes are given in Figure 10. In addition to metabolizing these substrates, each of these enzymes is noted for its broad range of overlapping substrate specificities. Consequently, experimental measurements of enzyme activity can lead to erroneous conclusions because the activity being determined usually reflects two or more related genes (*16,27,30*). For example, the benzo[*a*]pyrene hydroxylase activity represents a constitutive P-450 in the IIC subfamily, in addition to the TCDD-inducible enzyme encoded by *CYP1A1* in the P-450I subfamily (*16*). The importance of studying the regulation of these genes with a specific cDNA probe cannot be overemphasized.

It should be pointed out that the *Gt-1* genes believed to be a member of the [*Ah*] battery have not yet been identified with certainty. The glutathione transferase *Yp* and *Ya* genes are candidates for the [*Ah*] battery. As will be discussed below, the [*Ah*] battery Phase II enzyme activities are elevated in rat liver preneoplastic nodules and induced in normal liver by TCDD, 3-methylcholanthrene, and β-naphthoflavone. Although the glutathione transferase Yp subunit mRNA appears to be most markedly increased in preneoplastic nodules and hepatomas, the regulation of the glutathione transferase Ya mRNA in preneoplastic nodules appears to differ from one nodule to the next, whereas the Yb mRNAs are induced by 3-methylcholanthrene (*27*). On the other hand, a β-naphthoflavone-responsive positive regulatory element has been characterized in the 5′ flanking sequences of the Ya subunit gene (*27,28*).

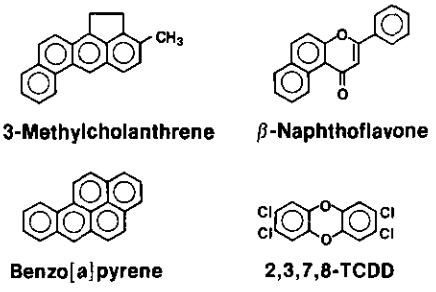

FIGURE 8. Chemical structures of four representative ligands for the Ah receptor (*16*).

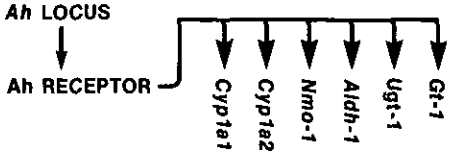

FIGURE 9. The six genes that have been defined as members of the [*Ah*] battery (*16*).

Phase I and Phase II Drug-Metabolizing Enzymes

Enzymes that metabolize drugs, phytoalexins, carcinogens, and other plant metabolites and environmental pollutants have classically been divided into two broad categories. Phase I enzymes are almost exclusively cytochromes P-450, which function by the insertion of one atom of atmospheric oxygen into a relatively inert substrate. Phase II enzymes act on the oxygenated intermediates, usually by conjugating them with various endogenous moieties (glucuronide, glutathione, sulfate) to produce extremely hydrophilic products that are easily excreted from the cell. Cytochromes P-450 are enzymes involved in the oxidative metabolism (biosynthesis as well as degradation) of steroids, fatty acids, prostaglandins, leukotrienes, biogenic amines, pheromones, and plant metabolites. These monooxygenases also metabolize countless drugs, chemical carcinogens and mutagens, and other environmental contaminants. The reactive intermediates formed by P-450 enzymes can be carcinogenic, mutagenic, and/or toxic. The coordinated regulation of Phase I and Phase II genes and the architectural arrangement of Phase I and Phase II enzymes in each cell are, therefore, critical factors ensuring metabolic clearance of foreign substances from the body with a minimal risk of accumulation of the oxygenated intermediates that might lead to cancer, mutation, or toxicity (*16,31*).

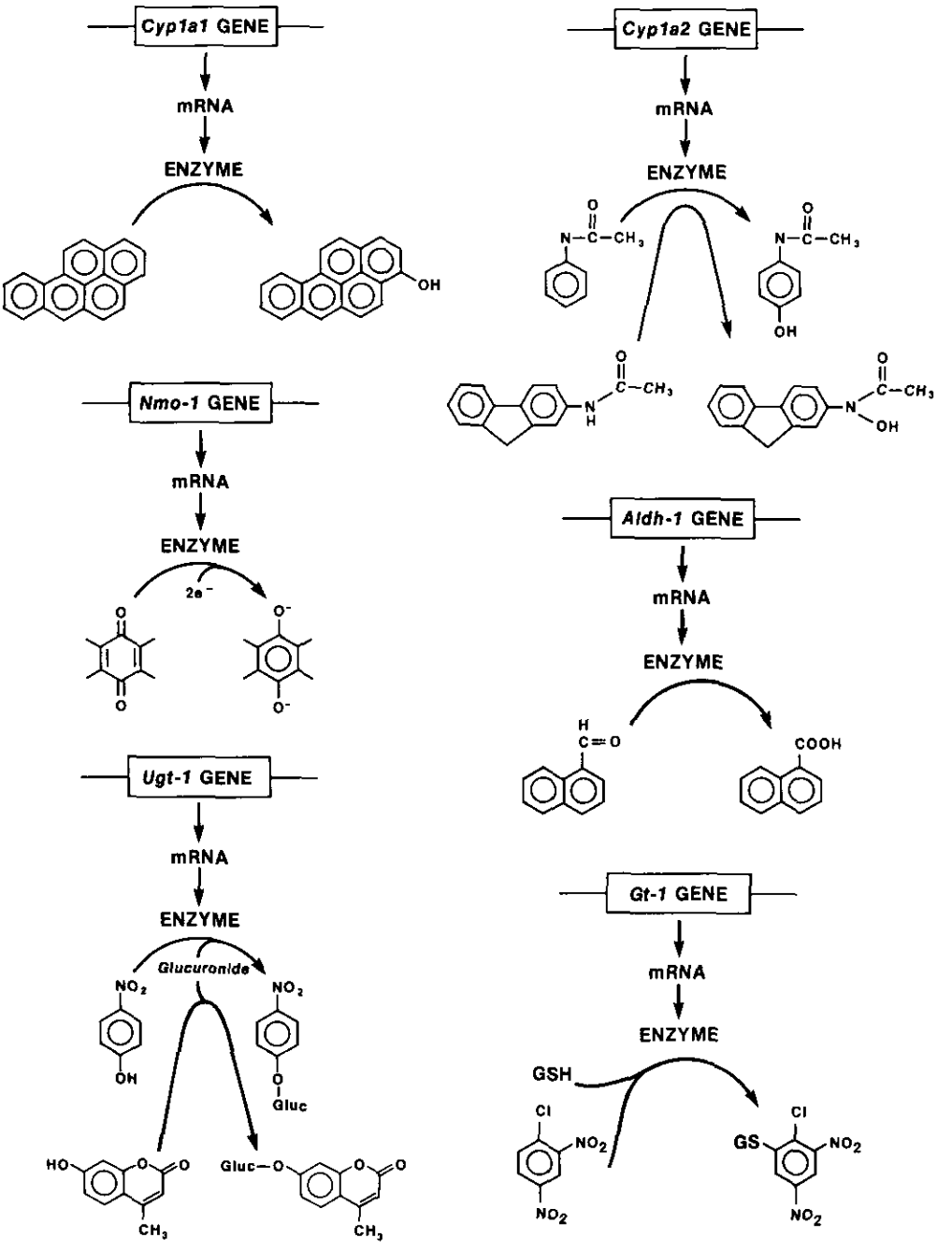

FIGURE 10. Representative catalytic activities encoded by the six genes of the [*Ah*] battery (*16*). Substrates include benzo(*a*)pyrene for *CYP1A1*, acetanilide and 2-acetylaminofluorene for CYP1A2, the two-electron reduction of numerous quinones for *Nmo-1*, naphthaldehyde for *Aldh-1*, *p*-nitrophenol and 4-methylumbelliferone for *Ugt-1*, and 1-chloro-2,4-dinitrobenzene for *Gt-1*.

It is important to emphasize that Phase II enzymes can operate independently of Phase I enzymes, in addition to being tightly coupled in some instances as described above. For example, the Phase II glucuronidation reaction shown in Figure 11 requires the Phase I P-450 reaction, whereas the Phase II quinone reduction shown in Figure 11 can occur with either the starting chemical or the Phase I metabolite as the substrate for *Nmo-1*. Just as the Phase I and Phase II enzymes are able to act on substrates independently, so we shall demonstrate below that the [*Ah*] battery Phase I genes can be regulated independently of the [*Ah*] battery Phase II genes.

CYP1A1 and *CYP1A2* Genes

The P-450 superfamily currently comprises more than 19 gene families, 10 of which exist in all mammals. A

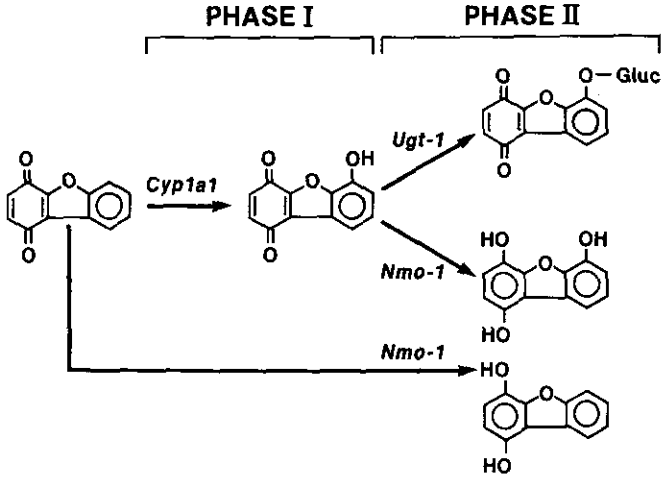

FIGURE 11. Examples of Phase I and Phase II metabolism with a hypothetical starting substrate (left).

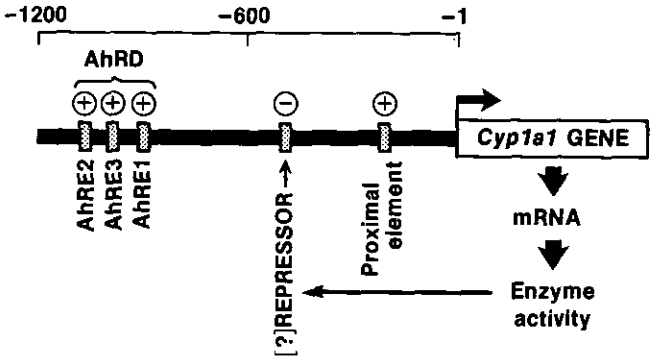

FIGURE 12. Diagram of the regulatory regions upstream from the mammalian *CYP1A1* gene. The precise location of the element participating in the negative autoregulatory loop remains to be determined. Although not indicated, there is a TATA box promoter less than 30 bp upstream from the mRNA cap site.

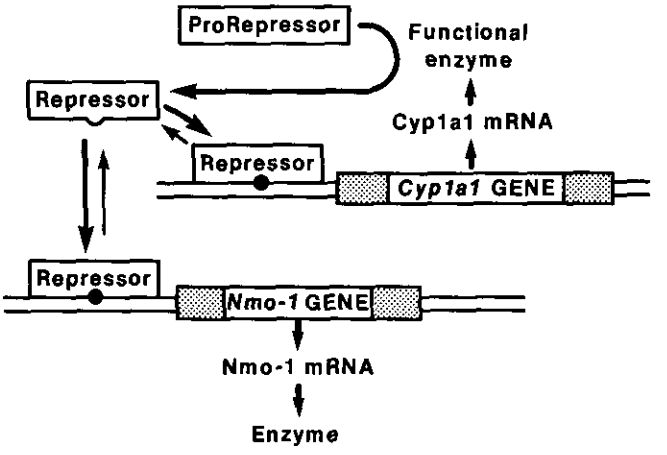

FIGURE 13. Suggested model in which the same repressor is common to both the Phase I *CYP1A1* gene and the Phase II *Nmo-1* gene. Modified and redrawn from Robertson et al. (*39*). It is now clear that this hypothesis is too simplistic.

nomenclature system based on evolution has been proposed (*32*). For naming a gene, the root symbol *CYP* (*Cyp* for mouse) denoting cytochrome P-450, is recommended; this is followed by a number designating the P-450 gene family, a letter indicating the subfamily, and an Arabic numeral representing the individual gene. Current estimates of the total number of functional P-450 genes in any one mammalian species range between at least 60 and perhaps more than 200. All of these genes are believed to have arisen by divergent evolution, i.e., from a common ancestral gene that existed probably more than 2.5 billion years ago. A major driving force for the large number of gene duplications is believed to be animal-plant warfare (*16,33*).

As mentioned above, *CYP1A1* and *CYP1A2* are the two Phase I genes defined as members of the [*Ah*] battery. *CYP1A1* and *CYP1A2* are the only two members of the P450I gene family (*32*). Little is known about mammalian *CYP1A2* gene regulation. Possibilities of control elements inside the gene and more than 2 kb upstream from the mRNA cap site have been suggested (*34,35*). Further discussion of *CYP1A2* gene regulation is beyond the scope of this review.

More is known about the regulation of *CYP1A1* gene expression than that of any other P-450 gene. A wealth of information has been gained through the use of plasmids containing mouse or rat *CYP1A1* upstream sequences and the chloramphenicol acetyltransferase reporter gene, sometimes with an heterologous promoter. These constructs have been transfected into mouse hepatoma Hepa-1 wild-type cell cultures, as well as *CYP1A1* metabolism-deficient (P^-1), receptorless (r^-), and chromatin binding-defective (cb^-) mutant lines (*16*).

As illustrated in Figure 12, about 1 kb upstream from the mRNA cap site lies an aromatic hydrocarbon-responsive domain (AhRD), comprising three aromatic hydrocarbon-responsive elements (AhREs) spaced approximately 80 bp apart (*36,37*). Gel mobility shift and methylation interference assays have demonstrated the interaction of a functional Ah receptor with the AhRD. Another element about 200 bp upstream from the cap site is absolutely essential for all constitutive and inducible *CYP1A1* gene expression; this proximal element is necessary but not sufficient for inducible gene expression, but interaction of the proteins that bind to the proximal element with those binding to the distal AhRD appears to be required for full *CYP1A1* induction by TCDD (*38*). Another element in the upstream *CYP1A1* regulatory sequences involves a negative autoregulatory loop; in the absence of a functional gene product in P^-1 cells, there is an apparent derepression of constitutive *CYP1A1* transcription (*16*).

Intriguingly, the *Nmo-1* gene is also transcriptionally activated in untreated P^-1 cells (*39*), suggesting a mechanism of common repression of [*Ah*] battery genes that requires a functional *CYP1A1* gene product (the enzyme) (Fig. 13). Transfection and expression of an exogenous *CYP1A1* cDNA in P^-1 cells represses the en-

dogenous CYP1A1 mRNA to constitutive levels seen in wild-type cells, suggesting further this intimate relationship between *CYP1A1* and *Nmo-1* gene regulation (A. Puga, B. Raychaudhuri, and D. W. Nebert, manuscript submitted).

Radiation Deletion Mouse Lines

Inbred mouse lines with overlapping radiation-induced chromosomal deletions involving the albino locus (*c*) on chromosome 7 have been examined, and indirect evidence has been found for regulatory genes located within the missing region of deletion homozygote mice (*40–46*). These regulatory loci appear to encode *trans*-acting factors that modulate the basal and inducible expression of genes that are located on other chromosomes. In most cases these genes are downregulated about 2- to 4-fold in the deletion homozygote (c^{14CoS}/c^{14CoS}), while the wild-type (c^{ch}/c^{ch}) and the deletion heterozygote (c^{ch}/c^{14CoS}) are unaffected (*42–46*). For example, the expression of glucose-6-phosphatase (*42*), tyrosine aminotransferase (Tat) and phosphoenolpyruvate carboxykinase (Pepck) induction by glucocorticoids (*44,45*), and metallothionein (*46*) is 2- to 4-fold decreased in the 14CoS/14CoS mouse, compared with the ch/ch and the ch/14CoS mouse, suggesting a gene on chromosome 7 that might encode one or more *trans*-acting positive regulatory factor(s). However, the expression of UDP-glucuronosyltransferase activity with *p*-nitrophenol as substrate (*40*) and glutathione transferase activity with 1-chloro-2,4-dinitrobenzene as substrate (*41*) was reported to be about 2-fold higher in untreated 14CoS/14CoS than in untreated ch/ch or ch/14CoS newborns. We believe that these two enzyme activities correspond to the *Ugt-1* and *Gt-1* genes, respectively (Fig. 10). The data thus suggest that a gene on chromosome 7 encodes a *trans*-acting negative regulatory factor that plays an important role in *Ah* battery regulation.

In the untreated 14CoS/14CoS mouse, we had expected to find the [*Ah*] battery Phase I and Phase II genes to be regulated in coordination, as appears to be the case in the untreated $P\bar{1}$ cell lines (*39*). However, *Nmo-1* gene activation, but not *CYP1A1* or *CYP1A2* gene activation, was found in untreated newborn 14CoS/14CoS mice (*47*). The constitutive expression of all three genes was nil in untreated newborn ch/ch and ch/14CoS mice. *Nmo-1* transcription was increased more than 12-fold, whereas mRNA levels were increased more than 100-fold in newborn 14CoS/14CoS mouse liver. These results indicate that the regulation of basal expression of the *Nmo-1* gene is distinct from that of the *CYP1A1* and *CYP1A2* genes. On the other hand, all three genes are inducible by TCDD in these mice (*47*), demonstrating that TCDD inducibility is independent of the chromosome 7-mediated mechanism of *Nmo-1* gene activation in untreated 14CoS/14CoS newborns. Recent work with the *Aldh-1* gene has shown that it behaves identically to the *Nmo-1* gene in $P\bar{1}$ cells and in the 14CoS/14CoS mice (D. D. Petersen and D. W. Nebert, manuscript in preparation).

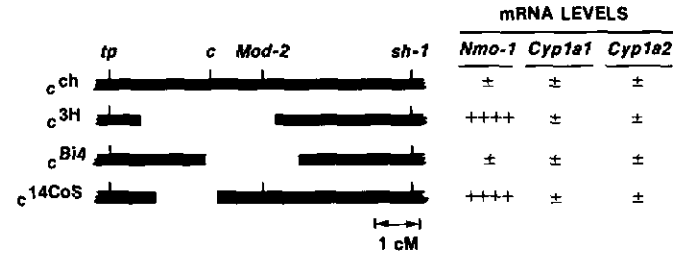

FIGURE 14. *Diagram of a small region around the albino (c) locus on chromosome 7, the approximate sizes of the deletions in the three mouse lines studied in this report [modified and redrawn from Gluecksohn-Waelsch (42) and Russell et al. (43)] and the levels of Nmo-1, CYP1A1 and CYP1A2 mRNA found in the untreated wild-type homozygote (c^{ch}/c^{ch}) and the three deletion homozygotes examined. (tp) Gene for taupe coat color; Mod-2 locus, mitochondrial malic enzyme; sh-1, gene for shaker-1. (±) Below level of detection; (+ + + +) > 100-fold increases.*

To define more precisely the chromosomal location of the putative *trans*-acting gene, we screened three mutant mouse lines having deletions that include the *c* locus (Fig. 14). *Nmo-1* mRNA was found to be markedly elevated in the untreated 3H/3H and the 14CoS/14CoS, but not the Bi4/Bi4 deletion homozygote. We therefore postulate that a novel gene encoding a putative *trans*-acting regulatory factor that negatively controls the *Nmo-1* gene is located on chromosome 7 within an approximately 1.1-cM region.

It has been estimated that, on the average, about 30 genes would be present in a region of 1.0 cM of genomic DNA (*48*). Therefore, we would expect that the 14CoS/14CoS deletion might comprise less than three dozen genes, with a range of perhaps one to 100 genes.

Regulatory proteins can have a positive effect on transcription under one set of circumstances and a negative effect under another (*49*). It is therefore conceivable, although quite unlikely, that the elevated expression of *Nmo-1* mRNA (*47*), *Aldh-1* mRNA, and *Ugt-1* and *Gt-1* enzyme activities (*40,41*), as well as the decreased expression of the four genes described above (*42–46*), might represent the action of the same gene that is missing in the 14CoS/14CoS deleted chromosomal region. We have indirect evidence suggesting that this is not, however, the case. Several SV40-transformed ch/ch, ch/14CoS, and 14CoS/14CoS mouse liver lines have been established and characterized; while the *Nmo-1* response in the 14CoS/14CoS cell lines is identical to that seen in 14CoS/14CoS newborn mouse liver, the Tat and Pepck response to dexamethasone is not (J. Chou, D. D. Petersen, and D. W. Nebert, unpublished). We therefore believe these data support the notion that the chromosome 7 gene encoding the *trans*-acting negative regulatory factor is different from that encoding the *trans*-acting positive regulatory factor.

Figure 15 illustrates our current hypothesis on the [*Ah*] regulatory pathways in the wild-type ch/ch mouse,

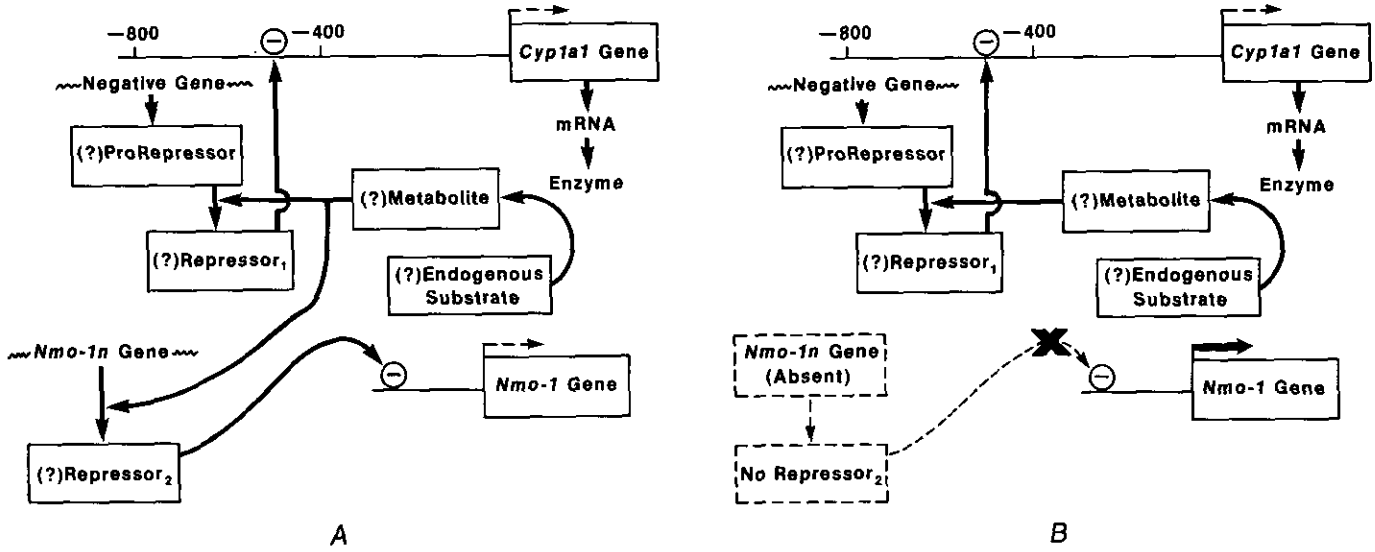

FIGURE 15. Proposed scheme for several interactive levels of regulation between the *CYP1A1* gene and the *Nmo-1* gene in (*A*) the wild-type ch/ch mouse and (*B*) the 14CoS/14CoS deletion homozygote mouse. The encircled minus signs denote putative negative control elements whose precise locations are not yet known. The putative endogenous substrate, metabolite, proRepressor, and repressor molecules (denoted by question marks) are not known. The *CYP1A1*, *Nmo-1*, and *Nmo-1n* genes have been mapped to mouse chromosomes 9, 8, and 7, respectively (*16,47*). Chromosomal location of the gene encoding the putative negative effector of *CYP1A1* transcription is not known.

as compared with that in the 14CoS/14CoS deletion homozygote. It should be emphasized that we began our search for a putative repressor gene acting on the expression of all [*Ah*] battery genes (Fig. 13). Instead, we have localized a putative repressor gene on mouse chromosome 7 that acts on the expression of [*Ah*] battery Phase II genes, but not [*Ah*] battery Phase I genes. Because studies of this chromosome 7 gene affecting *Aldh-1*, *Ugt-1*, and *Gt-1* gene expression are still preliminary, we propose the name *Nmo-1n* for the gene encoding a negative effector of *Nmo-1* gene transcription. The presence of two copies or one copy of the *Nmo-1n* gene on mouse chromosome 7 (Fig. 16) is sufficient

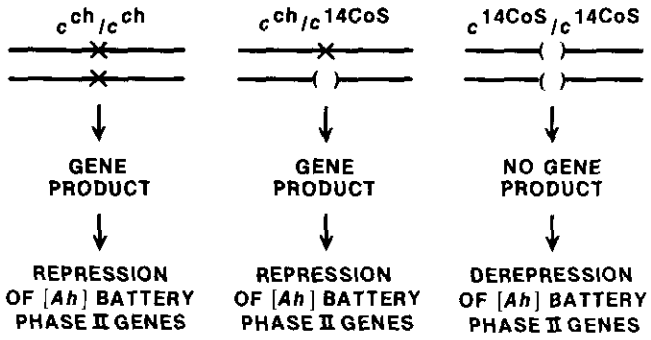

FIGURE 16. Diagram of the chromosome 7 regions in the three mouse genotypes. (X) Location of the putative *Nmo-1n* gene. The open area defined by parentheses denotes the radiation-induced deletion. With both copies of the *Nmo-1n* gene absent, there is the release from negative control of the [*Ah*] battery Phase II genes, which is independent of regulation of the [*Ah*] battery Phase I genes.

to repress the [*Ah*] battery Phase II genes. On the other hand, absence of both copies of the *Nmo-1n* gene leads to the putative derepression of the [*Ah*] battery Phase II genes (Fig. 16), in a mechanism that is independent of regulation of the [*Ah*] battery Phase I genes.

Rat Liver Preneoplastic Nodules

Rat liver preneoplastic nodules have been studied as a model system for tumor progression (*50*). The rats are fed one of several carcinogens, such as 2-acetylaminofluorene, followed by injury to the liver, such as carbon tetrachloride (cell death) or partial hepatectomy (decrease in organ cell number). These stimuli lead to the proliferation, or clonal expansion, of cells initially changed by a mutation. Only a small percentage of preneoplastic nodules actually proceed to frank malignancy. Interestingly, the *CYP1A1* and *CYP1A2* enzymes are undetectable, while the *Nmo-1*, *Aldh-1*, *Ugt-1*, and *Gt-1* enzyme activities in the preneoplastic nodules are elevated (*16,27,29,50,51*). It appears likely that these previous results with rat liver preneoplastic nodules are related to our recent observations that the murine [*Ah*] battery Phase II gene regulation is independent of the [*Ah*] battery Phase I gene regulation.

Michael Reaction Acceptors

Michael acceptors are potent electrophilic compounds with an electron-withdrawing group one carbon atom removed from an olefinic double bond (Fig. 17). In an intriguing report, Talalay and co-workers compared a series of Michael acceptors and found that the *Nmo-1*

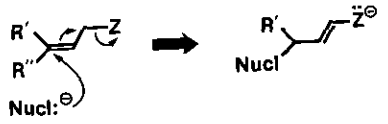

FIGURE 17. Mechanism by which an electrophilic Michael reaction acceptor interacts with an intracellular nucleophilic macromolecule [Nucl:$^{(-)}$]. (Z) An electron-withdrawing moiety.

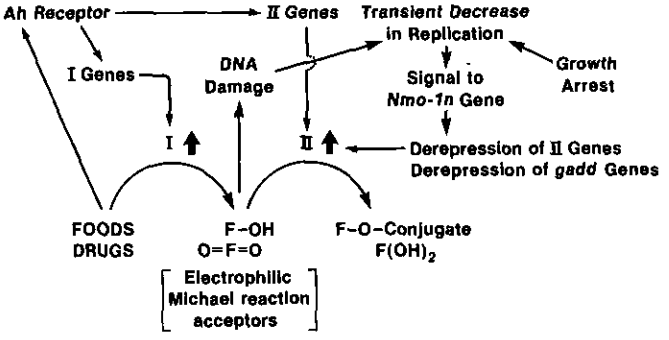

FIGURE 18. Illustration of the scheme in which [*Ah*] battery Phase I metabolism is suggested to be a prerequisite for the induction of [*Ah*] battery Phase II genes (*52*). It is now clear that this hypothesis is too simplistic.

inducing properties of several Michael reaction acceptors generally parallel the potency of the electron-withdrawing group (*52*). It was therefore postulated that Phase I metabolism is essential in order to produce metabolites that will induce Phase II genes (Fig. 18). However, the $P\bar{1}$ cells (*39*) have no *CYP1A1* or *CYP1A2* gene expression, and the untreated 14CoS/14CoS newborn mice (*47*) have no detectable *CYP1A1* or *CYP1A2* expression, yet *Nmo-1* and *Aldh-1* gene expression is markedly increased in both instances. Hence, in view of these results, we believe that Phase I metabolism *per se* is not essential for [*Ah*] battery Phase II gene induction.

DNA Damage and *Nmo-1* Induction

We have found that chemicals as diverse as menadione, butylated hydroxytoluene, and methylmethanesulfonate (MMS) especially at near-toxic concentrations, activate *Nmo-1* gene expression in Hepa-1 wild-type cells (D. D. Petersen, A. J. Fornace, Jr., K. Dixon, and D. W. Nebert, *manuscript in preparation*). One property these chemicals have in common is that they are all capable of causing DNA damage. In support of this hypothesis, the Nmo-1 enzyme has been found to activate 5-(aziridin-1-yl)-2,4-dinitrobenzamide to a new cytotoxic DNA crosslinking agent, 5-(aziridin-1-yl)-4-hydroxylamino-2-nitrobenzamide, which induces Nmo-1 mRNA in Walker rat carcinoma cells (*53*; John J. Roberts, personal communication). Moreover, 5-azacytidine and dietary methyl-group deficiency in mice induce Nmo-1 mRNA and certain glutathione transferase activities (*54*). In fact, the introduction of UV-damaged DNA alone into Hepa-1 wild-type cells in culture also activates *Nmo-1* expression (D. D. Petersen, A. J. Fornace, Jr., K. Dixon, and D. W. Nebert, manuscript in preparation). These data strongly suggest that the signal for *Nmo-1* gene activation can be mediated by way of DNA damage.

DNA Damage-Inducible Genes

If DNA damage induces the [*Ah*] battery Phase II genes as one subset, how many other classes of DNA damage-inducible genes might exist in the mammalian cell? To approach this question, hybridization subtraction was used to enrich UV-induced cDNAs of transcripts in Chinese hamster cells (*55*). Of 49 different cDNA clones characterized, most encode nonabundant transcripts that become rapidly induced 2- to 10-fold after UV irradiation. Two of the cDNA clones sequenced were metallothionein I and II, respectively, and another had significant homology with a rat DNA helix-destabilizing protein. Of 23 cDNA clones chosen for further study, none of the nucleotide sequences matches anything in the latest GenBank data base. These 23 genes, believed to represent single-copy genes, were categorized according to their response to various DNA-damaging agents (Table 1). Class I transcripts are induced by UV irradiation but not by the alkylating agent MMS. Class IIa transcripts are induced by both UV and MMS, whereas Class IIb transcripts are induced 2- to 6-fold by UV but 10- to > 25-fold by MMS and other alkylating agents. Interestingly, we found that the three Class IIb transcripts are markedly elevated in the untreated 14CoS/14CoS newborn mouse, but not in the untreated ch/ch or ch/14CoS newborn mouse (*56*).

Growth Arrest-Inducible Genes

DNA damage has distinct effects on the normal cell cycle. During typical cell growth, the following phases

Table 1. Induction of DNA damage-inducible transcripts by various agents.[a]

	Induction				
	Nucleotide excision		Base excision		Heat
cDNA probe	UV	AAAF	MMS	H_2O_2	shock
Class I (10 genes)	+	+	*0*	*0*	*0*
Class IIa (*10 genes*)	+	+	+	+	0
Class IIb (*3 genes*)	+	+	+ +	+ +	0
HSP70	0	0	0	0	+ +
β-Actin	0	0	0	0	0

[a] (+) Denotes 2- to 10-fold, (+ +) denotes 10- to > 25-fold. Exceptions: most of the UV induction was ≤ 6-fold, clone A15 (Class IIa) was heat shock-inducible (*55*). UV, ultraviolet irradiation; AAAF, *N-acetoxy-2-acetylaminofluorene*; MMS, *methylmethanesulfonate*; H_2O_2, hydrogen peroxide. Nucleotide excision and base excision refer to the types of DNA repair induced by UV (or AAAF) and MMS (or H_2O_2), respectively.

in the cell cycle have been described (Fig. 19): G_1, S (when DNA synthesis occurs), G_2, and M (when mitosis occurs). The G_0 phase denotes nongrowing cells that have left the cell cycle. DNA damage is known to delay progression through the cell cycle and entry into mitosis. In many cases, such delays probably represent active, cell-programmed processes and not simply the deleterious effects of DNA damage. For example, in *E. coli* a role in the inhibition of cell growth is played by the *sulA* gene, a member of the SOS regulon that is DNA damage-inducible (*57*). In yeast cells, the *Rad9* gene product is responsible for the arrest of cells in G_2 produced by X-irradiation, and this G_2 arrest is absent in the radiosensitive *rad9* mutant (*58*). There appear to be numerous check-and-balance functions that normally operate during the cell cycle, and these functions can be induced by DNA damage or other types of stress to the cell. For example, in human, rodent, Drosophila, slime mold, and fungal cells, the transient inhibition of DNA synthesis—seen after DNA damage—is decreased or absent in certain DNA damage-sensitive mutants (*58,59*).

We reasoned that a subset of DNA damage-inducible genes might be growth arrest-inducible. We therefore examined mRNA from growth-arrested cells by probing with each of the 23 genes listed in Table 1. Interestingly, five DNA damage-inducible transcripts, including the three IIb genes, are increased by several growth cessation signals: serum reduction, medium depletion, contact inhibition, and a 24-hr exposure to hydroxyurea. These five genes have been designated the *gadd* genes, for growth arrest and DNA damage inducible (*56*).

In conclusion, certain stimuli elicit the activation of the [*Ah*] battery Phase I genes, other stimuli the [*Ah*] battery Phase II genes, and still other stimuli the *gadd* genes (Table 2). These data are consistent with the present-day concept of modularity in promoters and enhancers (*60*). The promoters and enhancers that control transcription comprise multiple genetic elements, or modules. The cellular transcriptional machinery is able to gather and integrate the regulatory information conveyed by each module, and this information can be developmental-, sex- or tissue-specific. These integrated signals allow different sets (or batteries) of genes to evolve distinct, often complex, patterns of transcriptional regulation. It will be interesting to compare the regulatory elements of the [*Ah*] battery Phase I genes, the [*Ah*] battery Phase II genes, and the *gadd* genes.

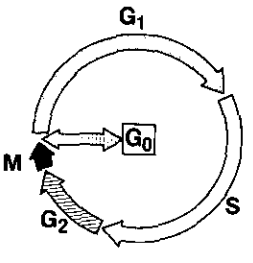

FIGURE 19. The normal cell growth cycle.

Table 2. Summary of the responses of three sets of genes to five experimental conditions.[a]

	TCDD	P_1-mutant	14CoS/14CoS mouse	MMS	Growth arrest
[*Ah*] battery	+ +	+ +	0	0	0
Phase I genes					
[*Ah*] battery	+ +	+ +	+ +	+ +	0
Phase II genes					
gadd genes	0	0	+ +	+ +	+ +

[a] (+ +) Denotes significant (≥ 2-fold) gene activation; (0) no significant gene activation.

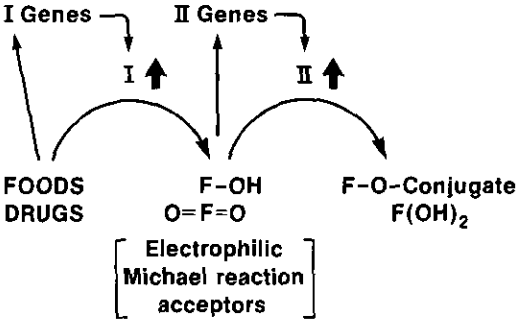

FIGURE 20. Hypothetical scheme by which [*Ah*] battery Phase I and Phase II genes and *gadd* genes respond to oxidative stress. (F) Chemicals in foodstuff.

Conclusions

In summary, a variety of inducers that bind to the Ah receptor are positive activators of [*Ah*] battery Phase I and Phase II genes (Fig. 20). Numerous oxygenated metabolites, including phytoalexins, cause DNA damage irrespective of whether or not Phase I metabolism occurs. Both DNA damage and growth arrest are known to produce a transient slowing in replication. It is somewhere herein that the *Nmo-1n* gene senses an unknown signal that the cell is in distress.

The *Nmo-1n* gene thus responds by releasing its negative control on the [*Ah*] battery Phase II genes and perhaps the *gadd* genes, thereby allowing all of these genes to become expressed. Hence, we conclude that the region of mouse chromosome 7 missing in the 14CoS/14CoS mouse contains a master switch gene that responds to environmental adversity, such as oxidative stress. This response is independent of Phase I (*CYP1A1* and *CYP1A2*) gene expression. Whether this region on chromosome 7 represents a single gene, *Nmo-1n*, controlling both the [*Ah*] battery Phase II genes and the *gadd* genes, or whether there are two or more genes controlling the [*Ah*] battery Phase II genes and the *gadd* genes will only be clarified after further work.

In eukaryotic cells, as in prokaryotes, there is likely to be a finite number of global mechanisms (batteries of genes activated in coordination) that respond to a nearly infinite number of adverse environmental stimuli. For example, the global responses of bacteria to DNA damage are known to include the H_2O_2-inducible

pathways (*1*), the adaptive response to alkylating agents (*2*), the heat shock system, mismatch repair (*3*), and the SOS regulatory system (*4*). The *OxyR*-regulated H_2O_2-inducible system (*1*) is clearly different from the [*Ah*] gene battery. The adaptive response appears to be limited to the induced synthesis of certain enzymes that repair alkylating lesions in DNA (*2*). Interestingly, the hypothesis involving the [*Ah*] gene battery illustrated in Figure 20 is quite similar to the *recA/lexA*-regulation of the prokaryotic SOS response shown in Figure 21.

It is well known that during the SOS response an identical set of pleiotropic effects is seen following a variety of stresses that damage DNA or interrupt DNA replication in *E. coli*. The responses to these stresses include an increased capacity for DNA repair, enhanced mutagenicity, growth arrest, filamentation of cells, cessation of respiration, DNA degradation, increases in stable DNA replication, and induction of some resident prophages (*60*). We are currently attempting to correlate these prokaryotic processes with their eukaryotic equivalents and to understand the role played by activation of the [*Ah*] battery Phase II and *gadd* genes.

The [*Ah*] gene battery appears to be involved in both apoptosis and the cellular response to oxidative stress. What do these two processes have in common? It remains to be determined whether TCDD-induced apoptosis (*18*) is caused by the induction of Phase I genes, the induction of Phase II genes, or another process beyond Phase I and Phase II gene activation. Apoptosis involves the programmed death of some cells, so that others might survive. Such a process normally involves endogenous signals and is seen in embryonic development, normal tissue turnover, and hormone-dependent atrophy. We suggest that a process similar or identical to apoptosis, involving exogenous signals (e.g., phytoalexins), might occur during tumor promotion (Fig. 6).

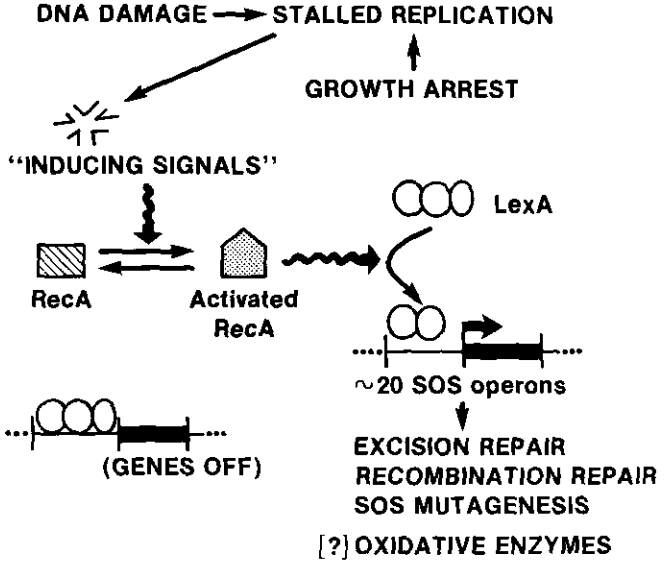

FIGURE 21. Diagram of the overall SOS response in prokaryotes. Modified and redrawn from Ossanna et al. (*4*).

In the face of chemical adversity, the primary drive for the eukaryotic or prokaryotic cell is to survive. In eukaryotic cells, it is clear that the [*Ah*] gene battery participates in this function.

This paper is dedicated to the memory of Ernst W. Caspari, 1910–1988. We thank our colleagues, especially Kathleen Dixon, for valuable discussions and the careful reading of this manuscript.

REFERENCES

1. Tartaglia, L. A., Storz, G., and Ames, B. N. Identification and molecular analysis of *oxyR*-regulated promoters important for the bacterial adaptation to oxidative stress. J. Mol. Biol. 210: 709–719 (1989).
2. Sekiguchi, M., and Nakabeppu, Y. Adaptive response: induced synthesis of DNA repair enzymes by alkylating agents. Trends Genet. 3: 51–54 (1987).
3. Walker, G. C. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. 48: 60–99 (1984).
4. Ossanna, N., Peterson, K. R., and Mount, D. W. Genetics of DNA repair in bacteria. Trends Genet. 2: 55–58 (1986).
5. Paxton, J. D. Phytoalexins—a working redefinition. Phytopathol. Zeitschr. 101: 106–109 (1981).
6. Dixon, R. A. The phytoalexin response: elicitation, signalling and control of host gene expression. Biol. Rev. 61: 239–291 (1986).
7. Ames, B. N. Mutagenesis and carcinogenesis: endogenous and exogenous factors. Environ. Mol. Mutagen. 14 (Suppl. 16): 66–77 (1989).
8. Berkley, S. F., Hightower, A. W., Beier, R. C., Fleming, D. W., Brokopp, C. D., Ivie, G. W., and Broome, C. V. Dermatitis in grocery workers associated with high natural concentrations of furanocoumarins in celery. Ann. Intern. Med. 105: 351–355 (1986).
9. Orrenius, S. Biochemical mechanisms of cytotoxicity. Trends Pharmacol. Sci. (FEST Suppl.): 1–4 (1985).
10. Thor, H., Mirabelli, F., Salis, A., Cohen, G. M., Bellomo, G., and Orrenius, S. Alterations in hepatocyte cytoskeleton caused by redox cycling and alkylating quinones. Arch. Biochem. Biophys. 266: 397–407 (1988).
11. Orrenius, S., McConkey, D. J., Jones, D. P., and Nicotera, P. Ca^{2+}-activated mechanisms in toxicity and programmed cell death. ISI Atlas Sci. Pharmacol. 319–324 (1988).
12. Nicotera, P., Thor, H., and Orrenius, S. Cytosolic-free Ca^{2+} and cell killing in hepatoma-1c1c7 cells exposed to chemical anoxia. FASEB J. 3: 59–64 (1989).
13. Wyllie, A. H., Kerr, J. F. R., and Cumi, A. R. Cell death: the significance of apoptosis. Int. Rev. Cytol. 68: 251–306 (1980).
14. Morris, R. G., Hargreaves, A. D., Duvall, E., and Wyllie, A. H. Chromatin cleavage in apoptosis: association with condensed chromatin morphology and dependence on macromolecular synthesis. Am. J. Pathol. 115: 426–436 (1984).
15. Wyllie, A. H. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. Nature 284: 555–556 (1980).
16. Nebert, D. W., and Gonzalez, F. J. P450 genes: structure, evolution and regulation. Annu. Rev. Biochem. 56: 945–993 (1987).
17. Evans, R. M. The steroid and thyroid hormones receptor superfamily. Science 240: 889–895 (1988).
18. McConkey, D. J., Hartzell, P., Duddy, S. K., Hakansson, H., and Orrenius, S. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin kills immature thymocytes by Ca^{2+}-mediated endonuclease activation. Science 242: 256–259 (1988).
19. Poland, A. P., Palen, D., and Glover, E. Tumour promotion by TCDD in skin of HRS/J hairless mice. Nature 300: 271–273 (1982).
20. Luster, M. I., Germolec, D. R., Clark, G., Wiegand, G., and Rosenthal, G. J. Selective effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and corticosteroid on in vitro lymphocyte maturation. J. Immunol. 140: 928–935 (1988).
21. Yuspa, S. H., and Poirier, M. C. Chemical carcinogenesis: from

animal models to molecular models in one decade. Adv. Cancer Res. 50: 25–70 (1988).
22. Moolenaar, W. H. Effects of growth factors on intracellular pH regulation. Annu. Rev. Physiol. 48: 363–376 (1986).
23. Grinstein, S., Rotin, D., and Mason, M. J. Na^+/H^+ exchange and growth factor-induced cytosolic pH changes. Role in cellular proliferation. Biochim. Biophys. Acta 988: 73–97 (1989).
24. Maly, K., Uberall, F., Loferer, H., Doppler, W., Oberhuber, H., Groner, B., and Grunicke, H. H. *Ha-ras* activates the Na^+/H^+ antiporter by a protein kinase C-independent mechanism. J. Biol. Chem. 264: 11839–11842 (1989).
25. Huot, S. J., Cassel, D., Igarashi, P., Cragoe, Jr., E. J., Slayman, C. W., and Aronson, P. S. Identification and purification of a renal amiloride-binding protein with properties of the Na^+-H^+ exchanger. J. Biol. Chem. 264: 683–686 (1989).
26. Nebert, D. W. The *Ah* locus: genetic differences in toxicity, cancer, mutation and birth defects. CRC Crit. Rev. Toxicol. 20: 153–174 (1989).
27. Pickett, C. B., and Lu, A. Y. H. Glutathione S-transferases: gene structure, regulation, and biological function. Annu. Rev. Biochem. 58: 743–764 (1989).
28. Daniel, V., Sharon, R., and Bensimon, A. Regulatory elements controlling the basal and drug-inducible expression of glutathione S-transferase Ya subunit gene. DNA 8: 399–408 (1989).
29. Jones, Jr., D. E., Brennan, M. D., Hempel, J., and Lindahl, R. Cloning and complete nucleotide sequence of a full-length cDNA encoding a catalytically functional tumor-associated aldehyde dehydrogenase. Proc. Natl. Acad. Sci. USA 85: 1782–1786 (1988).
30. Iyanagi, T., Haniu, M., Sogawa, K., Fujii-Kuriyama, Y., Watanabe, S., Shively, J. E., and Anan, K. F. Cloning and characterization of cDNA encoding 3-methylcholanthrene-inducible rat mRNA for UDP glucuronosyltransferase. J. Biol. Chem. 261: 15607–15614 (1986).
31. Schuster, I., Ed. Cytochrome P-450: Biochemistry and Biophysics. Taylor and Francis, London, 1989.
32. Nebert, D. W., Nelson, D. R., Adesnik, M., Coon, M. J., Estabrook, R. W., Gonzalez, F. J., Guengerich, F. P., Gunsalus, I. C., Johnson, E. F., Kemper, B., Levin, W., Phillips, I. R, Sato, R., and Waterman, M. R. The P450 gene superfamily. Update on the naming of new genes and nomenclature of chromosomal loci. DNA 8: 1–13 (1989).
33. Gonzalez, F. J., and Nebert, D. W. Evaluation of the P450 gene superfamily: animal-plant "warfare," molecular drive, and human genetic differences in drug oxidation. Trends Genet., in press.
34. Ikeya, K., Jaiswal, A. K., Owens, R. A., Jones, J. E., Nebert, D. W., and Kimura, S. Human *CYP1A2*. Sequence, gene structure, comparison with the mouse and rat orthologous gene, and genetic differences in liver 1A2 mRNA concentrations. Mol. Endocrinol. 3: 1399–1408 (1989).
35. Quattrochi, L. C., and Tukey, R. H. The human cytochrome *CYP1A2* gene contains regulatory elements responsive to 3-methylcholanthrene. Mol. Pharmacol. 36: 66–71 (1989).
36. Whitlock, Jr., J. P. The regulation of gene expression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Pharmacol. Rev. 39: 147–161 (1987).
37. Nebert, D. W., and Jones, J. E. Regulation of the mammalian cytochrome P_1450 (*CYP1A1*) gene. Int. J. Biochem. 21: 243–252 (1989).
38. Neuhold, L. A., Shirayoshi, Y., Ozato, K., and Nebert, D. W. Regulation of mouse *CYP1A1* gene expression by dioxin. Requirement of two *cis*-acting elements during the induction process. Mol. Cell. Biol. 9: 2378–2386 (1989).
39. Robertson, J. A., Hankinson, O., and Nebert, D. W. Autoregulation plus positive and negative elements controlling transcription of genes in the [*Ah*] battery. Chem. Scripta 27A: 83–87 (1987).
40. Thaler, M. M., Erickson, R. P., and Pelger, A. Genetically determined abnormalities of microsomal enzymes in liver of mutant newborn mice. Biochem. Biophys. Res. Commun. 72: 1244–1250 (1976).
41. Gatmaitan, Z., Lewis, S., Turchin, H., and Arias, I. M. Premature development of ligandin (GSH transferase B) in mice with an inherited defect in endoplasmic reticulum-Golgi structure and function. Biochem. Biophys. Res. Commun. 75: 337–341 (1977).
42. Gluecksohn-Waelsch, S. Genetic control of morphogenetic and biochemical differentiation: lethal albino deletions in the mouse. Cell 16: 225–237 (1979).
43. Russell, L. B., Montgomery, C. S., and Raymer, G. D. Analysis of the albino-locus region of the mouse: characterization of 34 deficiencies. Genetics 100: 427–453 (1982).
44. Schmid, W., Muller, G., Shutz, G., and Gluecksohn-Waelsch, S. Deletions near the albino locus on chromosome 7 of the mouse affect the level of tyrosine aminotransferase mRNA. Proc. Natl. Acad. Sci. USA 82: 2866–2869 (1985).
45. Loose, D. S., Shaw, P. A., Krauter, K. S., Robinson, C., Englard, S., Hanson, R. W., and Gluecksohn-Waelsch, S. *Trans* regulation of the phospho*enol*pyruvate carboxykinase (GTP) gene, identified by deletions in chromosome 7 of the mouse. Proc. Natl. Acad. Sci. USA 83: 5184–5188 (1986).
46. DeFranco, D., Morris, Jr., S. M., Leonard, C. M., and Gluecksohn-Waelsch, S. Metallothionein mRNA expression in mice homozygous for chromosomal deletions around the albino locus. Proc. Natl. Acad. Sci. USA 85: 1161–1164 (1988).
47. Petersen, D. D., Gonzalez, F. J., Rapic, V., Kozak, C. A., Lee, J. Y., Jones, J. E., and Nebert, D. W. Marked increases in hepatic NAD(P)H:menadione oxidoreductase gene transcription and mRNA levels correlated with a mouse chromosome 7 deletion. Proc. Natl. Acad. Sci. USA 86: 6699–6703 (1989).
48. Landegren, U., Kaiser, R., Caskey, C. T., and Hood, L. DNA diagnostics—molecular techniques and automation. Science 242: 229–237 (1988).
49. Adler, S., Waterman, M. L., He, X., and Rosenfeld, M. G. Steroid receptor-mediated inhibition of rat prolactin gene expression does not require the receptor DNA-binding domain. Cell 52: 685–695 (1988).
50. Farber, E. Cellular biochemistry of the stepwise development of cancer with chemicals: G.H.A. Clowes Memorial Lecture. Cancer Res. 44: 5463–5474 (1984).
51. Buchmann, A., Kuhlmann, W., Schwarz, M., Kunz, W., Wolf, C. R., Moll, E., Friedberg, T., and Oesch, F. Regulation and expression of four cytochrome P-450 isoenzymes, NADPH-cytochrome P-450 reductase, the glutathione transferases B and C, and microsomal epoxide hydrolase in preneoplastic and neoplastic lesions in rat liver. Carcinogenesis 6: 513–521 (1985).
52. Talalay, P., De Long, M. J., and Prochaska, H. J. Identification of a common chemical signal regulating the induction of enzymes that protect against chemical carcinogenesis. Proc. Natl. Acad. Sci. USA 85: 8261–8265 (1988).
53. Knox, R. J., Boland, M. P., Friedlos, F., Coles, B., Southan, C., and Roberts, J. J. The nitroreductase enzyme in Walker cells that activates 5-(aziridin-1-yl)-2,4-dinitrobenzamide (CB 1954) to 5-(aziridin-1-yl)-4-hydroxylamino-2-nitrobenzamide is a form of NAD(P)H dehydrogenase (quinone) (EC 1.6.99.2). Biochem. Pharmacol. 37: 4671–4677 (1988).
54. Wagner, G., Pott, U., Bruckschen, M., and Sies, H. Effects of 5-azacytidine and methyl-group deficiency on NAD(P)H:quinone oxidoreductase and glutathione transferase in liver. Biochem. J. 251: 825–829 (1988).
55. Fornace, Jr., A. J., Alamo, Jr., I., and Hollander, M. C. DNA damage-inducible transcripts in mammalian cells. Proc. Natl. Acad. Sci. USA 85: 8800–8804 (1988).
56. Fornace, Jr., A. J., Nebert, D. W., Hollander, C. M., Luethy, J. D., Papathanasiou, M., Fargnoli, J., and Holbrook, N. J. Mammalian genes coordinately regulated by growth arrest signals and DNA-damaging agents. Mol. Cell. Biol. 9: 4196–4203 (1989).
57. Lavin, M. F., and Schroeder, A. L. Damage-resistant DNA synthesis in eukaryotes. Mutat. Res. 193: 193–206 (1988).
58. Weinert, T. A., and Hartwell, L. H. The *RAD9* gene controls the cell cycle response to DNA damage in *Saccharomyces cerevisiae*. Science 241: 317–322 (1988).
59. Lee, M. G., Norbury, C. J., Spurr, N. K., and Nurse, P. Regulated expression and phosphorylation of a possible mammalian cell-cycle control protein. Nature 333: 676–679 (1988).
60. Dynan, W. S. Modularity in promoters and enhancers. Cell 58: 1–4 (1989).